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Clinicopathologic Features and Pathologic Diagnosis of Hepatitis E

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Abbreviations

FFPE: formalin-fixed, paraffin-embedded; gt: genotype; HEV: hepatitis E virus; IHC: immunohistochemistry; ORF= open reading frame.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Abstract

Infection with the hepatitis E virus (HEV) is one of the most common causes, if not the most common, of acute hepatitis worldwide. In the last decade, we have learned that in addition to the endemically and epidemically occurring form of hepatitis E, which is predominantly transmitted by contaminated drinking water, and constitutes a significant health problem in resource-poor countries, there is a globally existing form of hepatitis E, which is a zoonosis, and as such primarily transmitted by the consumption of contaminated meat products. Although in most cases hepatitis E is subclinical or mild and self-limiting, pregnant women and patients with liver cirrhosis may have severe, occasionally even fatal disease, and immunocompromised individuals may develop chronic hepatitis E. Considering the substantial global health burden caused by HEV infection, it is surprising how limited our knowledge of hepatitis E pathology still is. In this paper, we describe localization studies on HEV infection and discuss their implications for everyday diagnostics. Furthermore, we outline and discuss the spectrum of histologic changes, which can be found in HEV infection in various clinical contexts.

Mini review

Hepatitis E, caused by an infection with the hepatitis E virus (HEV), which belongs to the family Hepeviridae, is one of the most frequent, perhaps even the most frequent causes of acute hepatitis worldwide, and thus represents a major global health problem.(1, 2) The question of how pathologists come into contact with hepatitis E essentially depends on which part of the world they are practicing in.(1, 3)

HEV infection is widespread worldwide, but it manifests differently depending on the geographical region. Well documented has been a major epidemic that broke out in Delhi, India, in the mid-1950s which is now considered to be the first description of hepatitis E,(4) as well as another epidemic in Kashmir in the late 1970s.(5) In the early 1980s, a new virus was identified and cloned in connection with a major outbreak of unexplained hepatitis A virus (HAV)- and hepatitis B virus (HBV)-negative hepatitis in Afghanistan, which was subsequently referred to as hepatitis E virus (HEV).(6, 7) Later on, the above mentioned Delhi and Kashmir epidemics, respectively, could also retrospectively be classified as Hepatitis E. In countries in Asia, Africa and Central America, hepatitis E often occurs in this epidemic form. It is caused by genotypes (gt) 1 and 2, and mainly transmitted by contaminated drinking water. The clinical manifestation of HEV infection is very variable.(1) In most cases, the infection is asymptomatic, but can also be severe and even lethal.(8, 9) Pregnant women and patients with a pre-existing severe liver disease are particularly at risk of worse outcome.(5, 10)

A few years ago, our perception of hepatitis E started to change, after it had become obvious that, in addition to the epidemic form described above, there is another manifestation of hepatitis E which represents a zoonosis occurring globally including in particular also Europe and North America.(11-14) This autochthonous form of hepatitis E is caused by the worldwide occurrence of gt3, and in Asia by the additional occurrence of gt4. The main

reservoir for these viruses are pigs and wild boars.(1) The virus is transmitted mainly through the consumption of meat products that are not cooked sufficiently. What was surprising at that time was the realization that authochthonous HEV infection can also lead to chronic progressions in immunocompromised individuals,(15-18) which in this form had not been described before in the epidemic form.

At the molecular and cell biological level, we now have a solid understanding of central processes of HEV infection, in particular of the function of the three major proteins encoded by three different open reading frames (ORF). While the ORF1 protein (pORF1) is responsible for virus replication, the ORF2 protein (pORF2) is the capsid, which also forms the main antigenic structure and is used for vaccination. The ORF3 (pORF3) protein in turn is responsible for the release of viral particles from the cell.(2, 19)

Considering the frequency of hepatitis E worldwide and the large global health burden it represents, it is amazing how rudimentary the picture was until recently that we had of the course of infection in vivo, especially in the tissue context of the human liver. Insights into the life cycle of the virus could be gained in recent years by analyzing chimeric mice with human liver cells.(20-22) Although these models are suitable to study the life cycle in the 3D tissue context, and also allow to perform drug testing, they are limited in that the natural cellular and humoral human immune response cannot be recapitulated.(23, 24) Our own group has recently performed systematic analyses on the localization of HEV RNA and the three major HEV proteins (pORF1, pORF2 and pORF3) not only in the cell culture system but also in liver tissue of hepatitis E patients. Following this approach, we were able to perform in situ RNA hybridization and immunohistochemistry on formalin-fixed, paraffin-embedded (FFPE) tissue, allowing us to simultaneously localize HEV RNA and proteins directly in human livers, and semi-quantitatively describe the subcellular localization of the three proteins.(25) We made interesting observations especially for the HEV pORF2 protein. On the one hand, we were able to show that this protein is also found in the nucleus of infected hepatocytes, which

was surprising at the time, but has now been confirmed by other groups.(26) On the other hand, we were able to show that pORF2, unlike pORF1 and pORF3, can also be visualized very well in FFPE material of HEV-infected livers, so that the immunohistochemistry of pORF2 can also be used for diagnostic purposes. **(FIGURE 1 A)** This provides us a robust method for the histopathologic diagnosis of hepatitis E.(27, 28)

Immunohistochemistry for the presentation of HEV infection is a valuable ancillary test in everyday diagnostics, since the histologic changes that can occur in hepatitis E are very variable, thus making the histopathologic differential diagnosis of hepatitis E really challenging.(29) Compared to other viral hepatitis, there are few reports on histologic changes.(30) The histology of the epidemic form of hepatitis E has already been described in detail in the context of the above mentioned historical hepatitis epidemic in India in a substantial case number cohort of 90 biopsies, whereby a cholestatic form was distinguished from a non-cholestatic form.(31) In reports on the acute autochthonous form of hepatitis E with significantly smaller case numbers, changes similar to those observed in hepatitis A were described.(32-34) In summary, key histologic features of the acute hepatitis pattern are: Expanded portal tracts with a mixed infiltrate including lymphocytes, plasma cells and granulocytes, lobular disarray, Kupffer cell activation, variable degree of (mostly spotty) hepatocyte necrosis, hepatocyte swelling and ballooning degeneration, cholestatic rosettes, canalicular and cytoplasmic bilirubinostasis, biliary epithelial damage, and cholangitis.(30, 35) **(FIGURE 1 B)**

In reports on persistent / chronic courses of HEV infection in immunosuppressed patients, histologic changes similar to those in other forms of chronic hepatitis were described, including the development of portal-emphasized chronic inflammation and fibrosis.(15, 36) Furthermore, cases with almost only reactive changes(36) or a prominent cholangitic component(37) have been described. In summary, key histologic features of the chronic hepatitis pattern are: Portal-based mononuclear inflammation (predominantly lymphocytes),

interface activity possible, variable degree of hepatocyte necrosis (mostly single scattered apoptotic hepatocytes), and variable degree of fibrosis possible.(30) **(FIGURE 1 C)**

The above mentioned compilation of reports on hepatitis E-associated histopathologic changes, supplemented by our own observations,(29, 35) shows how heterogeneous the histopathologic findings in hepatitis E are. Thus, it becomes clear that these changes overlap extensively with histologic changes observed in other diseases. For the acute, self-limiting form of hepatitis E, these are mainly: Hepatotropic viruses other than E, non-hepatotropic viruses, autoimmune hepatitis (AIH), drug-induced liver injury and toxins as well as metabolic diseases (e.g. Wilson's disease). For the chronic form of hepatitis E, these are mainly: Hepatotropic viruses other than E, non-hepatotropic viruses, autoimmune hepatitis (AIH), drug-induced liver injury,(38) and in the context of transplantation also T-cell mediated rejection in (liver) transplant patients, graft versus host disease (GvHD) in hematologic stem cell transplanted patients, and recurrence of viral hepatitis.

In a special situation are patients with pre-existing (severe) liver disease in whom an additional hepatitis E infection is more difficult to detect clinically and histopathologically against the background of the pre-existing disease.(39-41) **(FIGURE 1 D)** It is therefore given that differential diagnosis is extremely challenging, which is made even more difficult by the fact that the clinical pictures also overlap. Therefore, the correlation with the clinical context, which is generally recommended in hepatopathology, is especially important for hepatitis E.

In summary, immunohistochemistry for HEV pORF2 has become a robust and helpful tool for histopathologic diagnostics. The fact that hepatitis E associated histologic changes cover a very broad spectrum ranging from a nearly normal liver histology, over acute and chronic hepatitis patterns to (sub)-total liver necrosis, together with the notion that no pathognomonic or even characteristic changes could be defined so far (and probably do not exist), makes the histopathologic diagnosis very challenging. Since the manifestation is different depending

on the clinical context, it is advisable to consider this for the differential diagnosis of hepatitis E in everyday life.

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FIGURE LEGEND

Fig. 1. Histopathologic findings observed in liver biopsies from patients with HEV infection: (A) HEV-pORF2 immunohistochemistry with geographically distributed positive areas and different staining patterns: e.g. predominantly cytoplasmic (upper insert) or predominantly nuclear (lower insert). (B) Acute hepatitis pattern with severe lobular disarray, hepatocyte damage and cholestasis. (C) Chronic hepatitis pattern with portal-based mononuclear inflammation, some interface activity, single scattered apoptotic hepatocytes (arrow head) and development of fibrosis (insert Sirius red). (D) Acute on chronic liver damage pattern with signs of nutritive-toxic damage, inflammation and cirrhosis (insert Sirius red). Scale bars 250 μm for H&E overviews in A-D and Sirius red in C-D; scale bar 25 μm for inserts in A and B.

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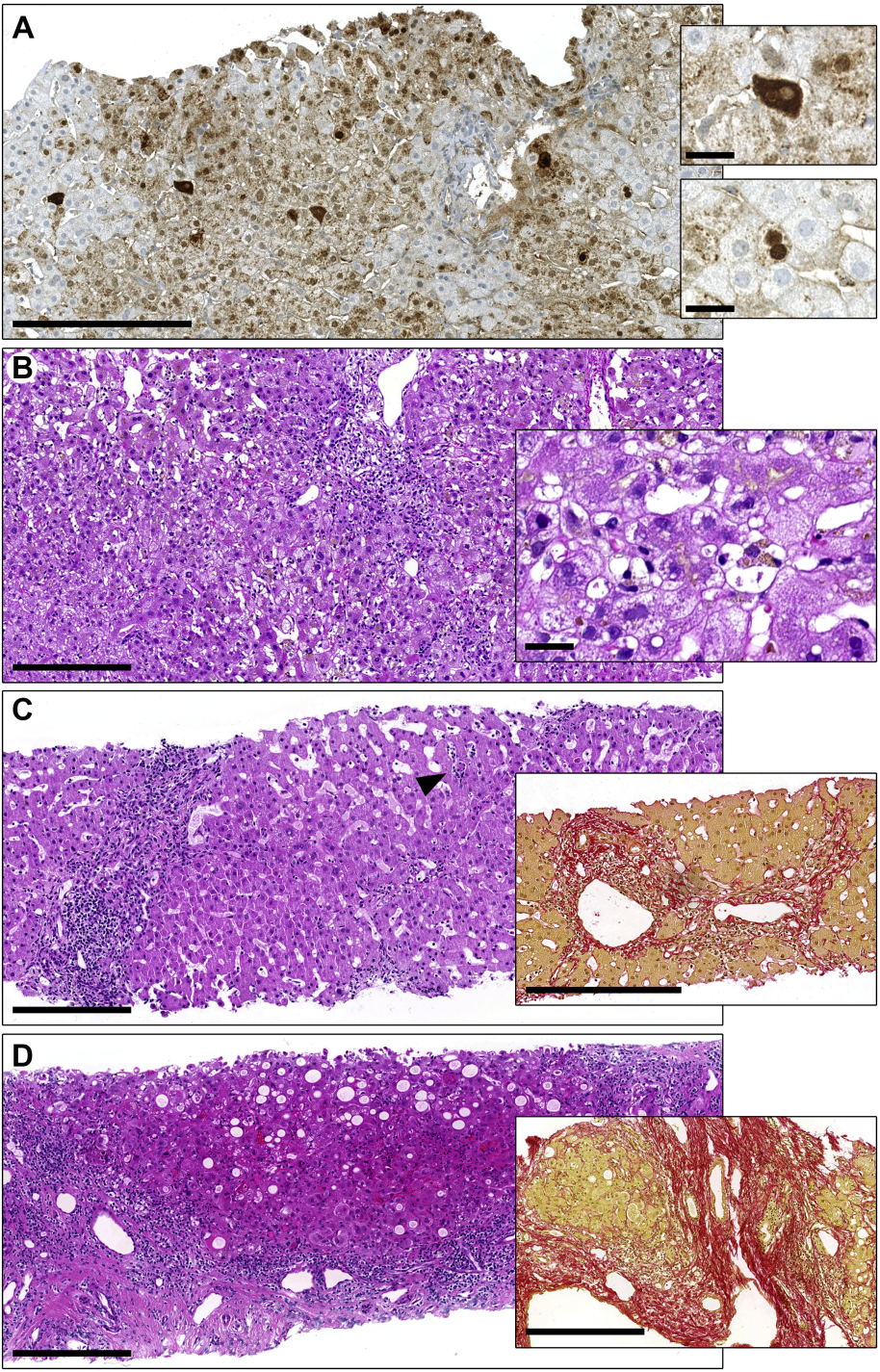

Figure 1